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AXL, A TYROSINE KINASE RECEPTOR, IS INVOLVED IN ANGIOGENESIS

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Angiogenesis, is a multistep process consisting in the sprout of new blood vessels from pre-existing ones, that is essential in many patho-physiological processes [1]. Endothelial cells (EC) play a key role in angiogenesis, secreting many factors involved in this process. Vascular endothelial growth factors (VEGFs) family, is a very important angiogenetic factor, responsible of the blood vessels growth and differentiation in the embryo and in other physiological conditions, as in several angiogenesis dependent diseases [2].
Axl is a tyrosine kinase receptor (RTK) that belongs to a subfamily of RTK with Mer and Rse in virtue of their structure consisting of two amino terminal immunoglobulin-like domains and two fibronectin-type III domains in the extracellular region and a distinctive intracellular kinase domain [3-5]. GAS6, a protein encoded by the growth arrest specific gene 6 [6], is a common ligand for Axl, Mer and Rse [7]. Gas6 and Axl are both produced by EC, and are involved in many processes involving cellular adhesion, like cell-cell adhesion [8] or polymorphonuclear cells adhesion to endothelium [9].
Aim of this work was to evaluate a possible role of Axl and its ligand GAS6 on angiogenesis. We evaluated the effects of GAS6 on EC migration, tubulogenesis and new vessels formations in an “in vivo” assay, the chick embryo chorioallantoic membrane assay (CAM) [10]. Chemotaxis assay was performed using a 48-well Boyden chamber, in the presence or not of GAS6 (1-800 ng/ml) and/or VEGF-A (20 ng/ml). Analysis of cellular migration showed that GAS6 not conditioned spontaneous EC migration, but inhibits VEGF-A mediated migration (n=4). Tubulogenesis was evaluated with Matrigel assay at a concentrations range of 20-100 ng/ml of GAS6 that inhibits tubulogenesis at concentrations of 80-100 ng/ml (n=3). To confirm these data in an in vivo assay, we performed experiments on CAM, and we verified that in a range of concentration of 1-20-200-800 ng/CAM, GAS6 inhibits new vessels formations mediated by 40 ng/CAM of VEGF-A, with a maximum of inhibitions at 20 ng/CAM. We analysed the pattern of Axl phosphorylation in EC stimulated with GAS6, performing an immunoprecipitation assay with an anti-Axl antibody, and we observed Axl phosphorylation induced by GAS6 stimulation in a range of concentration between 1 and 800 ng/mL of GAS6 and in a time range between 5 and 40 minutes. These data suggest an inhibitory role of the Axl /GAS6 interaction on angiogenesis.

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